

High-Performance Insect Diets

Relying on insects that prey on crop pests can have a serious drawback: By the time predator numbers become large enough to do a top-rate control job, the pests have already started taking an economic toll on the crop. But it's been prohibitively expensive to rear massive numbers of predators in captivity for release into fields at the most opportune time. Rearing enough host insects to satisfy the predators' voracious appetites, after all, doesn't come cheap.

That's why ARS scientists are searching for artificial diets that will satisfy the palates of beneficial insects, such as the spined soldier bug, the two-spotted stink bug, the big-eyed bug, and the minute pirate bug.

Simply providing a cheap diet that will keep the beneficial insects alive is not the final answer, says Thomas A. Coudron, a chemist at the ARS Biological Control of Insects Laboratory, in Columbia, Missouri. The diets must also help the insects thrive and produce multitudes of offspring fit for the field.

Coudron and his colleagues have been combining their expertise in insect biochemistry and physiology to improve the prospects for using beneficial insects. Currently sold mostly to organic gardens and greenhouses, these helpful bugs already make up a \$750-million-per-year business. But releasing them in large numbers to help protect field crops—not just organic gardens and greenhouses—will only make sense if the cost of rearing the insects can be significantly decreased, says Coudron.

ARS has already made progress toward this goal. Captive-reared lacewings, *Chrysoperla carnea*, once required an insect-egg diet costing more than \$300 per pound. By 1997, ARS had



ALLEN COHEN (K9249-1)

Above: *Lygus lineolaris* nymph feeds on broccoli during a feeding test. The insect, raised on an artificial diet, is tested to make sure it will feed on natural food sources before it's released.

Right: Insect weight and egg production are two of the main measures of diet performance. Research aide Parvaneh Khosravi prepares to weigh insects reared on one of several diets being tested.

PEGGY GREB (K9248-1)



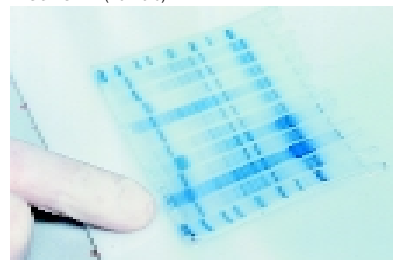
Technician Patrick Crittenden (seated) and entomologist Allen Cohen, discuss PAGE gel of hemolymph (blood) proteins from *Lygus* bugs. The protein banding in the gel Crittenden is pointing to (enlargement at far right) tells them the physiological age of the insect.

PEGGY GREB (K9245-1)



developed a lacewing diet that could be produced for \$2.50 per pound. The artificial diet, invented by entomologist Allen C. Cohen, now at the ARS Biological Control and Mass Rearing Research Unit, Mississippi State, Mississippi, also works for the big-eyed bug, *Geocoris punctipes*. This predator and the

PEGGY GREB (K9245-9)



lacewing have voracious appetites for a wide range of plant-feeding insects, such as whiteflies, aphids, scale insects, moth eggs and larvae, and mealybugs.

Cohen and his colleagues at Mississippi State also developed

biochemical and biological tests that commercial insectaries can use to quickly and accurately determine whether or not insects reared on artificial diets are able to devour large quantities of pest insects.

Artificial diets are also being developed to feed captive host pests. These insects are used in mass-rearing of beneficial predators and parasites. Cohen is trying various combinations of a basic recipe, which consists mainly of cooked chicken eggs, lima bean meal, wheat germ, soy flour, yeast, vitamins, and preservatives.

"The diets differ from one another in their nutritional quality," says Cohen. To determine which recipe gets the blue ribbon, the researchers measure differences in the pests' weight, longevity, biomass accumulation, and fecundity.

The scientists have proven that there is a correlation between a better diet and an increase in egg production. They used tests such as colorimetry, electrophoresis, and enzyme-linked immunosorbent assay (ELISA) to measure protein in the insect eggs. ELISA is based on the ability of an antibody to recognize and bind to a specific antigen, thereby identifying yolk proteins in the adult females and the eggs.

Test results were clear. One example is a diet for *Lygus hesperus*, a destructive bug that devours cotton plants. *L. hesperus* bugs reared on a diet of chicken egg and a plant product, rather than chicken egg and a meat paste, had more yolk proteins in their hemolymph and eggs. This measurement is predictive of a healthier insect and an adult with higher egg-producing capacity.

"This means that a simple immunological/biochemical test can be used early in the rearing process to predict that a diet or other rearing condition is good for or harmful to the insects," says Cohen. This translates into fewer insect losses and significant cost savings for producers.

By linking research in the areas of physiology, biochemistry, genetics, nutrition, and digestion, Cohen hopes to further improve the formulation and long-term storage of artificial diets—with the ultimate goal of making mass-rearing efficient and economical.

Coudron is also working on part of an immunological/biochemical test that would link to a method developed by ARS entomologist Terrance S. Adams to score the reproductive potential of spined soldier bugs and two-spotted stink bugs. The spined soldier bug feeds on more than 50 species of insects, and both predators feed on the Colorado potato beetle, a major pest of potatoes and related plants. Adams is at the Red River Valley Agricultural Research Center, Fargo, North Dakota. By tracing the biochemistry and physiology of egg production, he and Coudron are looking for ways to alter the diet, perhaps for different production stages, to tune up the bugs' nutritional, endocrine, and reproductive systems.

PEGGY GREB (K9247-9)



The fine-tuning may result in an increase in fertile egg production in insectaries that will far exceed the reproductive capacity of beneficial insects in nature, says Coudron. That's because of a tradeoff: Faced with fluctuating food supplies in the wild, adults may be programmed to enhance their chances for survival by producing fewer eggs.

The study of dietary effects on reproduction could help lead to year-round production of beneficial insects and their eggs. This would allow more efficient use of insectaries and encourage commercial investment in them.—By **Ben Hardin** and **Jesús García**, ARS.

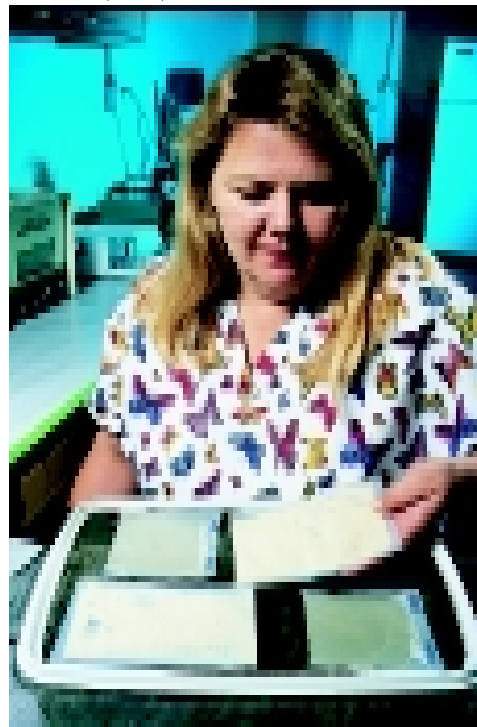
This research is part of Crop Protection and Quarantine, an ARS National Program (#304) described on the World Wide Web at <http://www.nps.ars.usda.gov>.

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PEGGY GREB (K9247-1)



Special insect diets are contained in the larger white packets being inspected by entomologist Gay McCain. The smaller packets contain eggs from insects reared on the diets.

Far left: Carrageenan gel pack containing several thousand *Lygus lineolaris* eggs from adults reared on the new diet developed by Allen Cohen.

Epidemiological studies have repeatedly shown that populations whose diets include plenty of fruits and vegetables have lower rates of cancer, heart disease, and other “ailments of aging.”

That’s why fruits and vegetables are high on the list of recommended foods in the *Dietary Guidelines for Americans*.

Scientists are keenly interested in learning which substances make fruits and vegetables so healthful. During the last decade, their curiosity has introduced us to a whole new language of phytonutrients, the beneficial compounds in plant foods. At the forefront of this inquiry are chemists in ARS’s Food Composition Laboratory at Beltsville, Maryland. Their collaboration with a sister laboratory has resulted in two phytonutrient databases.

In 1998, ARS’ Nutrient Data Laboratory, the group responsible for maintaining the national nutrient

databank, launched a database giving levels of various carotenoids—such as beta carotene or lycopene—in plant-based foods, thanks to the analytical expertise of researchers in ARS’ Food

Finessing the Flavonoids

Composition Laboratory. In 1999, a database of the isoflavones—like genistein and daidzein—in soy foods went online. You can view these databases at: <http://www.nal.usda.gov/fnic/foodcomp/Data/index.html>.

Now, the two laboratories are readying a database of flavonoids—the largest class of phytonutrients—for its electronic debut. ARS chemists Howard M. Merken and Gary R. Beecher developed what they hope will be “a universal system to

measure the promising flavonoids in all plant foods,” says Beecher. Until now, he says, chemists have had to tailor their flavonoid analyses to different types of foods. He expects the new system will be adopted by university scientists and commercial laboratories.

Western diets provide from several milligrams to a gram of flavonoids each day. The list of their health-giving properties is lengthy and growing. Various flavonoids have been shown to prevent oxidation, chelate (bind) metals, stimulate the immune system and also reduce an allergic response, prevent formation of carcinogens, impede cancer cell growth, and protect against bacteria and viruses.

Getting Them All With One Shot

Merken explains that foods contain more than 50 flavonoids, and they fit into 5 major subclasses: anthocyanidins, catechins, flavanones, flavones, and flavonols (see list). He says several

KEITH WELLER (K9263-1)



While monitoring the process of raspberry, blueberry, and strawberry flavonoid extractions, technician Casandra Merken checks the solvent level of the blueberry mixture.

Some Food Sources of Flavonoids

Anthocyanidins (cyanidin and delphinidin)

berries, grapes, fruit skins, and true fruit juices

Catechins (catechin and epicatechin)

true teas (not herbal teas)

Flavanones (hesperetin and naringenin)

citrus

Flavones (apigenin and luteolin)

grains and herbs

Flavonols (myricetin and quercetin)

fruits, onions, and botanicals

methods exist for identifying and measuring one or two, maybe even three, of the subclasses. But mixed diets contain flavonoids from all five subclasses. Most of the food flavonoids have glucose or some other sugar attached.

The system Merken developed with Beecher finds and separates the 18 most common food flavonoids, representing all 5 subclasses. The trick in making one system work for all the subclasses, says Merken, was to remove the attached sugars while the flavonoids are being removed from the foods. Most of the current methods measure the flavonoids with their sugars. But commercial standards—pure compounds of known quantity—aren't available for several of the flavonoid glycosides, as the sugar-coated flavonoids are called. The standards are necessary to calibrate the system and ensure accuracy.

Merken says a big hurdle in ensuring accuracy was to account for the progressive loss of some of the flavonoids in the boiling acid needed to extract them from the food. That's especially true for the anthocyanidins—the red and blue pigments that give berries, grapes, and other fruit skins their visual appeal. But it also happens to flavonols—the best known being quercetin, which is abundant in onions.

Recognizing a pattern in the way these compounds degraded in the boiling acid, Merken turned to a textbook he had used while teaching freshman chemistry.

"It was pseudo first-order kinetics," he says, "so I could use the same type of math that is used to measure the rate of radioactive decay—the math used in carbon dating.

"We don't lose catechins and flavanones during extraction because the solvents we use are much less destructive," he says. "And the flavones have proved very stable." Except for differences in the extraction method among the flavonoid subclasses, the chromatography is the same. "We use the same

KEITH WELLER (K9262-2)

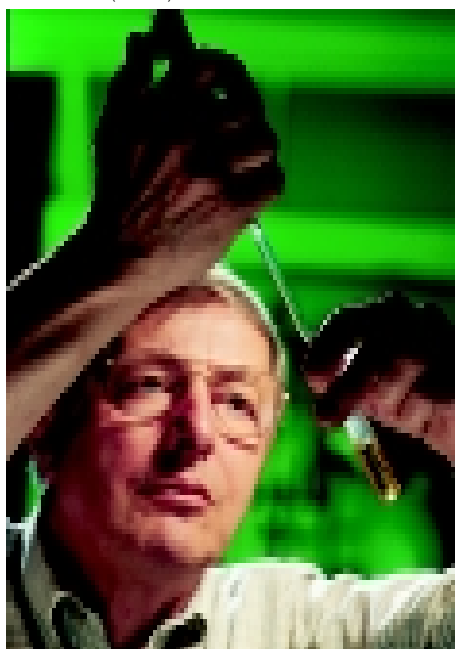


Chemist Howard Merken analyzes blueberry extract for flavonoids on a high-performance liquid chromatograph.

high-performance liquid chromatography system to measure all of them," says Merken.

After the system is fine-tuned, Beecher says, it will be able to analyze several foods in one day. He and Merken are using it to provide many of the values in the new flavonoid database. They are analyzing 50 commonly eaten fruits, vegetables, nuts, and other foods sampled by the Nutrient Data Laboratory. The project is partially funded by the National Heart, Lung and Blood Institute

KEITH WELLER (K9264-2)



Chemist Gary Beecher prepares a raspberry sample for HPLC analysis.

and the Produce for Better Health Foundation, which sponsors the 5-A-Day program for the fruit and vegetable industry, urging us to eat at least five fruits and vegetables daily.

The foods have been selected from grocery chains around the country by use of a statistically based sampling plan to ensure that they accurately represent the U.S. food supply, says Joanne M. Holden, who heads the Nutrient Data Laboratory. Meanwhile, her group is gathering and evaluating flavonoid data that has already been published in the scientific literature or supplied by the food industry. Acceptable data will be combined with new data being generated by Merken and Beecher. Holden expects the flavonoid database to be available in late 2001.

Beecher also wants the flavonoid data ready for an expert panel being sponsored by the National Academy of Sciences' Food and Nutrition Board. The panel will look at the body of research on some of the emerging phytonutrients to assess the importance of these components to health.

"Epidemiologists need data on all of these food components to draw associations between intake and health status," he notes.—By **Judy McBride, ARS.**

This research is part of Human Nutrition, an ARS National Program (#107) described on the World Wide Web at <http://www.nps.ars.usda.gov>.

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